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Survey

Control of interferon signaling in human papillomavirus infection

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Abstract

Human papillomaviruses (HPV) infect mucoal and cutaneous epithelium resulting in several types of pathologies, most notably, cervical cancer. Persistent infection with sexually transmitted oncogenic HPV types represents the major risk factor for the development of cervical cancer. The development of HPV-associated cervical cancer has been closely linked to the expression of the viral oncogenes E6 and E7 in the tumor cells. The major viral oncoproteins, E6 and E7, target the cellular tumor suppressor gene products p53 and Rb, respectively. As detailed within, these interactions result in the stimulation of proliferation and the inhibition of apoptosis, thus representing major oncogenic insults to the infected cell. In addition to mediating transformation, the E6 and E7 genes also play significant roles in altering the immune response against infected cells by suppressing interferon (IFN) expression and signaling. At the clinical level, IFNs have been used in the treatment of HPV-associated cervical intraepithelial neoplasia (CIN) or cervical cancers with mixed results. The success of the treatment is largely dependent on the subtype of HPV and the immune response of the patients. Despite this inefficiency, the increasing knowledge about the regulation of IFN signaling pathways at molecular level may hold a promise for the use of new therapeutic strategies against HPV infection. Studies on the regulation of the function of IFN-inducible gene products by the E6 and E7 may lead to the development of new therapeutic approaches based on strategies that modify the function of the HPV oncoproteins and restore IFN-signaling pathways through endogenous control mechanisms.

Contents

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1. Human papillomaviruses

Human papillomaviruses (HPVs) are small double stranded DNA viruses, which infect cutaneous and mucosal epithelium resulting in a variety of clinically important conditions, most notable cervical cancer [1]. The incidence of cervical cancer in North America is relatively uncommon due to the effectiveness of screening programs that assess cervical cytology by the Papanicolaou smear. However, on a global scale, cervical cancer is a major cause of mortality, especially in developing countries where screening programs are not routinely performed [2]. Precursor lesions for cervical cancer are known as cervical intraepithelial neoplasia (CIN). The observation that CIN occurs at a younger age than does invasive disease is consistent with the long latency period required to develop cervical cancer, which can take over 10 yr. Therefore, cervical cancer is a preventable disease if CIN lesions are detected early.

The epidemiology of cervical cancer has always suggested that it is sexually transmitted disease [3–5] implying an etiological role for an infectious agent. There is now no doubt that the infectious agent involved in cervical neoplasia is HPV and infection with the so-called oncogenic or high risk HPV types 16, 18, 31, 33, 35, 39, 41–45 is the major risk factor for this disease. However, the available epidemiological evidence also shows that that HPV infection alone is not sufficient for neoplastic progression. Thus only a fraction of individuals infected with high-risk viruses go on to develop CIN or cervical cancer. About 30% of CIN cases will progress to cervical cancer [6]. The HPV genome is usually present in an episomal configuration during infection and CIN, whereas in invasive cervical cancer, the genome is commonly integrated into the host DNA [7]. The only viral genes which are consistently expressed following integration is the E6 and E7 oncogenes and these genes are critical for the development of malignant transformation and also play a role in altering the cellular response to cytokines.

2. Transforming activity of the HPV oncoproteins

The genome of HPVs contains eight genes including the E6, E7, E5, E4, E2, E1, L1 and L2 genes. The early (E) genes are predominantly involved in regulating the viral life cycle and the late (L) genes encode the viral capsid proteins. The major viral transforming genes are the E6 and E7 genes, which target the cellular p53 and Rb genes respectively [8–10]. This review will therefore focus on the biological activity of the E6 and E7 genes.

The oncogenic potential of the E6 and E7 proteins from the high-risk HPVs is due predominantly to the ability of these viral proteins to target and inhibit the activity of the cellular p53 and Rb tumor suppressor proteins respectively. Since p53 and Rb induce apoptosis and control cell-cycle progression respectively, their inactivation by E6 and E7 represents a major carcinogenic insult to the infected cell. The continued expression of E6/E7 is necessary to sustain the proliferation and anti-apoptotic character of cervical cancer derived cells [11–13]. The ability of HPV to target both Rb and p53 allows the virus to stimulate cell replication, which also results in the replication of the viral genome while inhibiting apoptosis in the infected cell. Moreover, it has also been revealed that E6 and E7 may play a role in maintaining viral episomal DNA stability during infection [14].

3. The transforming activity of E6 and inhibition of apoptosis

The E6 proteins contain approximately 150 amino acids depending on the HPV type with an apparent molecular weight of about 18 kDa. Due to the low levels of this protein in the cell and a lack of good antibodies, it has been difficult to determine its location within the cell. Our own recent observations show that the oncogenic E6 proteins are equally distributed between the nucleus and the cytoplasm, whereas E6 from the non-oncogenic HPV types are predominantly cyto-

plasmic (unpublished observations). This is consistent to what has been previously reported for the oncogenic HPV E6 proteins [15,16]. It is also possible that the location of the E6 protein could change depending on the differentiation status of the cell or in response to different extracellular stimuli.

As mentioned above, a major target of oncogenic type E6 is the p53 tumor suppressor protein and this results in the degradation of p53 via the ubiquitin proteolytic pathway [17,18]. This observation provides a logical explanation why the p53 tumor suppressor gene is seldom mutated in HPV-positive cervical tumors [19,20]. In comparison, the p53 gene is mutated in the majority of other cancer types [21]. Cells expressing E6 lose the G1 checkpoint presumably due to loss of p53 [22] and are resistant to p53-mediated apoptosis [23–25]. The degradation of p53 by oncogenic type E6 is dependent upon a cellular protein termed E6-AP (E6-Associated Protein) [18,26]. There has however not been any link demonstrated to date between E6-AP and p53 in the absence of E6.

The ability of E6 to mediate p53 degradation is very important considering that p53 places a zero tolerance on cellular abnormalities through mediating apoptosis and preventing cell proliferation [27,28]. Therefore in order for tumor cells or viral oncogene expressing cells to survive, the p53 tumor suppressor protein must be lost through gene mutation or viral protein interaction. In the case of oncogenic HPVs, E6 is responsible for mediating the degradation of p53 thus ensuring the survival of the infected cell [14].

Another important activity of E6 with respect to mediating cellular immortalization and subsequent transformation is its ability to activate cellular telomerase activity [29]. The telomerase enzyme is important to maintain the stability of the chromosome ends (telomeres) during multiple rounds of cell proliferation. It is however unclear what role the telomerase enzyme may play in the normal virus life cycle.

A number of other cellular targets for E6 have also been identified and these may also contribute to the oncogenic activity of this protein. For example, the E6/E6-AP complex has also been implicated in the degradation of the apoptosis inducing c-Myc [30] and Bak proteins [31]. E6 from HPV-16 can also associate with the transcriptional co-activators CBP and p300 [32,33]. Since CBP/p300 is involved in the transcriptional activity of p53, this association also reduces the transcriptional transactivation activity of p53. Oncogenic HPV E6 has also been shown to mediate the degradation of the discs large (Dlg) tumour suppressor proteins, which have been shown to have tumor suppressor protein activity in *Drosophila* [34]. Dlg proteins may function as components of signal transduction pathways transmitting growth inhibitory signals from regions of cell–cell contact to downstream effectors which block cell proliferation and migration [35]. Taken together, E6 may have multiple activities in addition to inhibiting p53 and further studies are needed to define these interactions with respect to cell transformation.

4. The transforming activity of E7 and induction of cell cycle progression

As with E6, expression of E7 is necessary for the continuing proliferation of HPV containing cervical cancer cell lines [11,36,37]. The E7 proteins from the various HPV types contain approximately 100 amino acids and, similar to E6, contain a zinc binding motif in the C-terminal region [38,39]. The E7 protein is normally phosphorylated by casein kinase II [40] and has been located in a variety of cellular locations including the cytoplasm, the nucleus, and the nucleoli [41–44]. The HPV E7 shares homology with other DNA tumor virus proteins including Adenovirus E1A and SV40 large T and has been shown to interact with many of the same cellular targets including the Rb, p107, and p130, cyclin A, cyclin E, and AP-1 [44–48]. Given the nature of these cellular targets, these interactions suggest that a major role for E7 is to disrupt normal cell cycle control. Several studies have shown that expression of E7 is associated with increased cellular DNA synthesis [49–51]. The increase in DNA synthesis and cell proliferation is largely as a result of the interaction between E7 and the Rb family of proteins including Rb, p107 and p130. These interactions interfere with the ability of the Rb family to suppress the transactivating activity of the E2F transcriptional transactivators. E2F responsive promoters include dihydrofolate reductase, thymidine kinase, cdc 2, cyclin A, cyclin D, and DNA polymerases [52]. Expression of these genes is required for DNA synthesis and progression through the cell cycle and expression of E2F alone has been shown to induce DNA synthesis [53]. Therefore, E7 perturbs the normal control of cell proliferation by inhibiting the function of Rb resulting in the activation of E2F mediated transcription resulting in the induction of gene products which stimulate DNA synthesis and cell proliferation. It has also been shown that E7 destabilizes Rb and the related p107 protein potentially through the ubiquitin proteasome pathway [54,55]. The crystal structure of a C-terminal region 9 amino acid E7 peptide bound to Rb has now been defined [56].

In addition to targeting Rb and related proteins, E7 has also been shown to interact with the other cell cycle inhibitors including p21 [57,58] and p27 [59]. Clearly the interaction and suppression of these cell cycle inhibitors contribute further to the ability of E7 to stimulate cell proliferation through disrupting key cell cycle control molecules.

5. Synergism between E6 and E7 in inducing cell proliferation and inhibition of apoptosis

As mentioned above, both E6 and E7 bind to a number of cellular proteins, which play key roles in regulating cell cycle and apoptosis. It is noteworthy that inactivation of Rb results in the induction of E2F-mediated transcription, which in turn leads to the expression of the p14 ARF protein that stabilizes p53 by inhibiting MDM2-mediated p53 degradation [28]. Consequently, expression of oncogenes with E7-like activity can effectively induce p53-mediated apoptosis. Therefore, it is essential that HPV counteract the E7-mediated apoptosis through the above pathway by targeting p53 for degradation with the E6 protein. A summary of the functions of E7 and E6 in inducing cell proliferation and inhibiting apoptosis is shown in Fig. 1. Under these conditions, the viral genome is thus allowed to replicate and ultimately produce new viral particles in infected terminally differentiated cells.

6. HPV infection and regulation of immune system

Persistence of cutaneous and genital-HPV-induced lesions is common suggesting that infections can be sustained for an extended period without the induction of an effective immune response [60]. This also suggests that HPVs have evolved mechanisms for subverting the activation of immune response. Limiting exposure of viral gene products to the host immune system is an important evasion mechanism for these viruses [61]. First, there is no viremic phase during the life cycle, so the systemic immune system is avoided. Second, very low levels of viral proteins are expressed in the basal and spinous layers of epidermis, where they would be most likely to be recognized by Langerhans cells and infiltrating lymphocytes. Thirdly, extensive production of viral proteins takes place in terminally differentiated layers of the epidermis where shedding of the virus from this surface limits the exposure of assembled virions to the host immune system.

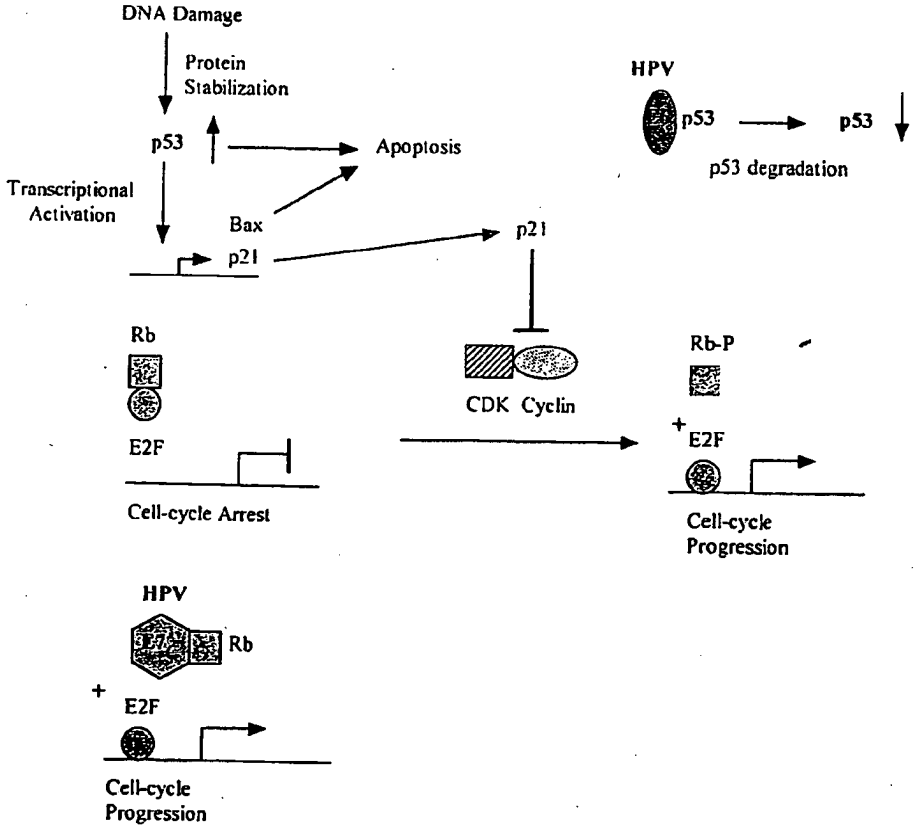

Fig. 1. Biochemical and biological functions of E6 and E7 proteins: DNA damage leads to phosphorylation and stabilization of p53. Activated p53 induces the transcription of genes involved in cell cycle arrest (e.g. the CDK inhibitor p21) or apoptosis (e.g. Bax). The E6 oncoprotein blocks p53 activation by degradation through the proteasome pathway. The E7 oncoprotein binds to the hypophosphorylated form of Rb in the so-called "pocket domain", which is partially conserved among the other Rb-related proteins. The effect of this interaction is to release free the transcription factor E2F, which is now able to activate the transcription of genes required in G1/S cell-cycle progression.

Table 1
Efficacy of IFNs in the treatment of HPV-associated disease

Disease	HPV subtype	Efficacy	References
IFN-α			
Cervical intraepithelial neoplasia			
CIN of lower genital tract	16	Effective	[99]
CIN	6/11/16/18	Partial	[100]
Cervical cancer	N/A	Partial	[101]
CIN	16	Partial	[102]
HPV-infected cervix	N/A	Partial	[103]
CIN2	N/A	No effect	[104]
Other genital infections			
Condylomata acuminata	6/11	Effective	[105]
Buschke-Lowenstein tumor	6/11	Effective	[106]
Vulvar vestibulitis	16/18	Effective	[107]
Condylomata acuminata	6	Effective	[108]
Intravaginal warts	N/A	Effective	[109]
Infected lower genital tract	N/A	Effective	[110]
Genital condyloma acuminatum	N/A	Effective	[111]
Genital warts	N/A	Effective	[112]
Chronic vulvodynia	N/A	Partial	[113]
Vestibular papillomatosis	N/A	Partial	[114]
Cervical condyloma	11/16	Partial	[115]
Genital warts	16/18	Partial	[116]
Condylomata acuminata	16/18	Partial	[117]
Microcondylomatosis	N/A	Partial	[118]
Genital HPV-infections	6/11/16/18	No effect	[119]
Laryngeal lesion			
Laryngotracheal papillomatosis	6/11	Effective	[120]
Laryngeal papilloma	6	No effect	[121]
Respiratory tract papillomas			
Respiratory tract papillomas	6/11	No effect	[122]
IFN-β			
Cervical intraepithelial neoplasia			
CIN grade II	N/A	Effective	[123]
CIN	N/A	Effective	[124]
CIN-II	N/A	Effective	[125]
CIN	N/A	Effective	[126]
CIN I, II & III	N/A	Effective	[127]
CIN	N/A	Effective	[128]
CIN	N/A	Partial	[129]
CIN I & II	N/A	Partial	[130]
Other genital infections			
Genital condylomata acuminata	N/A	Effective	[131]
Intraepithelial genital lesion	N/A	Effective	[132]
Genital HPV infections	N/A	Effective	[133]
Severe vulvar vestibulitis	N/A	Effective	[134]
Cervical & vaginal lesions	N/A	Effective	[135]
Genital condylomatosis	N/A	Effective	[136]
Genital warts	2/6/11	Partial	[137]
HPV-infected dysplasia	N/A	Partial	[138]
Lower female genital tract	N/A	Partial	[128]
Vulva infection	N/A	No effect	[139]
IFN-γ			
Cervical Intraepithelial neoplasia			
CIN	High risk	Effective	[140]
Cervical carcinoma	16	Effective	[141]
Other genital infections			
Condylomata acuminata	6	Effective	[142]
Bowenoid papulosis (BP)	16	Effective	[143]
Genital warts	All types	Effective	[144]

The expression of major histocompatibility complex (MHC) class I- and MHC class II-restricted T-cell responses are important for the control of most viral infections [62]. However, it appears that HPVs have evolved specific mechanisms to prevent their proteins. particularly those expressed in the lower layers of the epithelium, from being efficiently recognized by T cells. This is possibly mediated by producing viral proteins that are not efficiently presented as part of MHC complexes. Alternatively, HPV may have developed mechanisms that directly interfere with MHC expression and function as has been described for other DNA virus such as adenovirus, herpesvirus and pox viruses [62]. For example, interferon (IFN) stimulates expression of MHC class I and II as well as LMP2 and -7, which are part of the 20S proteasome degradation machinery used for the display of antigens to the cell surface [63–65]. Therefore, the inhibition of IFN sig-

naling by the HPV oncoproteins (see below) may be one of the mechanisms utilized by the virus to evade immune surveillance.

7. The efficacy of IFN treatment in HPV infection and associated disease

IFNs are a family of biological response modifiers that exhibit antiviral, antiproliferative and immunomodulating functions [66]. They consist of two different subtypes: type I IFNs (i.e. IFN-α/β) that are produced in virally infected cells, and type II IFN (i.e. IFN-γ) that is not virus inducible and is restricted to mitogen or cytokine-activated lymphoid cells such as T lymphocytes and natural killer (NK) cells [66]. In addition to the establishment of an antiviral state in uninfected cells, the elimination of virally infected cells is also critical in the host defense. In this context, type I IFNs exhibit a selective induction of apoptosis in virally infected cells and play a bifunctional role in limiting the spread of virus and eliciting an antiviral state in uninfected cells while promoting apoptosis in infected cells [67].

The treatment of HPV-infection and associated disease with IFNs has been under clinical experimentation. Theoretically, IFN treatment should result in the clearance of visible HPV lesions and elimination of the virus, even in cases of latent infections. However, despite the satisfactory results in some of the clinical studies, IFN treatment is still far from widespread therapy against HPV infection [68]. Studies on the effectiveness of IFN therapy in patients with CINs and cervical carcinomas have produced very inconsistent results and the conclusion regarding their efficacy has been controversial [69]. A compilation of data from various studies in vivo indicates that IFN-β is more effective than IFN-α and generally type II IFN (i.e. IFN-γ) more effective than type I (Table 1).

The molecular basis for this discrepancy in the efficacy of IFN treatment has not been determined, but it has been observed that expression of viral oncogenes, particularly E7, is significantly higher in the no responders than in responders [70]. Downregulation of HPV viral replication [70] and viral gene expression could be the reason for the positive clinical outcomes observed with IFNs [71–73]. However, studies with various cervical cancer cell lines in vitro showed that IFNs are unable to suppress HPV expression universally [69]. Thus, the efficacy of IFNs may be dependent upon the levels of expression of the HPV oncogenes, the distinct functions of the viral proteins in regulating IFN production and signaling, the complex interactions between the viral oncoproteins and cellular factors that affect both viral and host gene expression and function, and/or mutations unique to the infected cells that disrupt intracellular surveillance and regulatory mechanisms upon HPV infection [69].

8. IFN signaling and the Jak–Stat activation pathway

The mechanisms by which IFN transmit signals to the cell interior have been extensively studied in the recent years [74]. Type I IFNs (i.e. IFN-α/β) transduce their signals through the sequential activation of receptor associated *Janus* tyrosine kinases Jak1 and Tyk2 leading in tyrosine phosphorylation and activation of the signal transducers and activators of transcription Stat1 and Stat2 [74]. Activated Stat1/Stat2 heterodimers then translocate to the nucleus, where they associate with a 48 kDa DNA-binding protein [known as IFN stimulatory gene factor ISGF-3γ or IFN regulatory factor IRF-9 [75]] to form an active complex (known as ISGF-3) on the interferon response element (ISRE) [74] (Fig. 2). This element is known to mediate the induction of a number of functionally important IFN-stimulated genes (ISGs) including the translation initiation eIF-2α kinase PKR, 2′-5′ oligoA synthase, Mx, ISG-15 [74].

Type II IFN (i.e.IFN-γ) transduces its signals through the sequential activation of receptor associated Jak1/Jak2 and then Stat1. Activated Stat1 homodimers translocate to the nucleus and directly bind to members of the GAS (IFN-γ activated sequence) family of enhancers (Fig. 2). This element is known to mediate the induction of a distinct family of genes including IRF-1, IFP-53. For both types of IFN, Stat1 activation is mediated by a single phosphorylation site on tyrosine 701 [76]. In addition, phosphorylation of Stat1 on serine 727 is also required for maximal transcriptional activation [76]. Consistent with these observations, Stat1 null mice exhibit profound defects in IFN signaling, rendering them highly susceptible to infection with viruses and other pathogens [77,78].

9. Molecular actions of E6 and E7 in signaling induced by virus infection and IFNs

Several studies in vitro have demonstrated the ability of HPV oncoproteins to control signaling pathways that lead to the expression of IFNs and IFN-inducible genes. For example, it has been reported that HPV-16 E6 binds to the carboxyl-terminal domain of transcription factor IRF-3 and inactivates its transactivating function [79] (Fig. 3). IRF-3 is a member of the interferon regulatory factor (IRF) family whose members play a critical role in the regulation of the IFN α and β genes [80]. IRF-3 was originally characterized as a transcriptional factor that binds to ISRE. IRF-3 is part of a virus activated transcription factor complex includ-

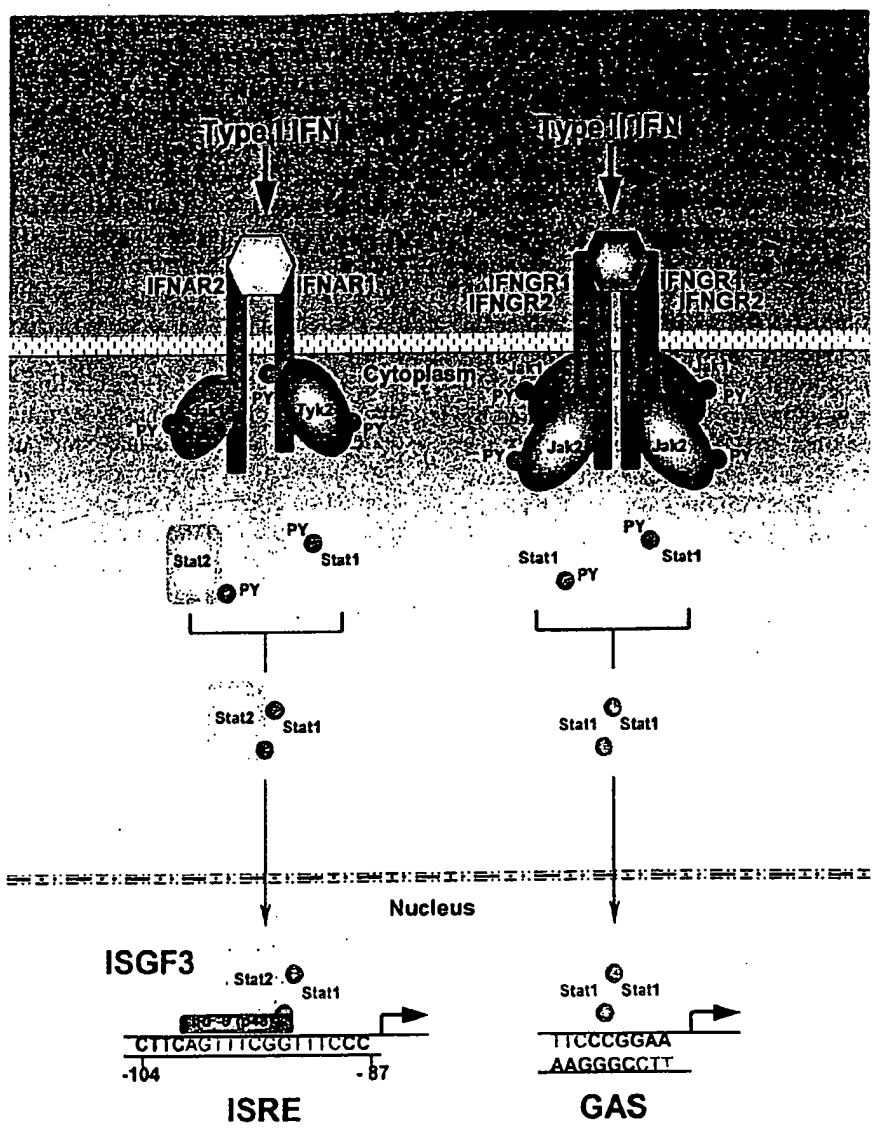

Fig. 2. A schematic model of activation of the Jak–Stat pathway by IFN: Binding of IFNs to their receptors results in the activation of the cytoplasmic tyrosine kinases of the *Janus* kinase (Jak) family, Jak1 and Tyk2 for IFN-α/β, and Jak1 and Jak2 for IFN-γ. Activated Jaks become autophosphorylated and subsequently phosphorylate the receptor on specific tyrosine residues, which function as docking sites for the *src* homology 2 (SH2) domain of Stat1 and Stat2. Tyrosine phosphorylation by Jaks results in heterodimerization of Stat1 and Stat2 or homodimerization of Stat1 and their nuclear translocation upon IFN-α/β or IFN-γ treatment, respectively. Stat1/Stat2 heterodimers bind to ISRE DNA sequence in the presence of a 48 kDa protein known as ISGF-3γ or IRF-9 to form the ISGF3 complex whereas Stat1 homodimers bind to GAS directly.

ing IRF-7, p300 and pCBP, whose activity is induced in response to viral infection [81]. Expression of HPV-16 E6 in human keratinocytes was able to diminish the induction of IFN-β gene expression by Sendai virus and consequently the expression of IFN-inducible genes [79]. Interestingly, inhibition of IRF-3-mediated transactivation was not observed with the benign HPV-6 E6 whereas the malignant HPV-18 E6 exhibited a modest binding to IRF-3 [79]. These finding indicated that inactivation of IRF-3 by E6 is probably specific for the HPV-16 type. Although IRF-3 is subjected to proteasome dependent proteolysis after virus infection this property is not modulated by E6 [79]. Considering the complex regulatory transcription pathways implicated in IFN-β gene transcription it is reasonable to speculate that E6 may interfere with other transcriptions factors involved in this process. Inasmuch as IRF-3 transactivation requires its interaction with CBP/p300 [81], inactivation of these co-activators by E6 [32,33] may also play a role in the inhibition of IFN-β gene transcription.

Another mechanism by which HPV infection impairs the antiviral and antiproliferative actions of IFN-α was reported by our group and involves the interaction of E6 with the tyrosine kinase Tyk2 [82]. We showed that expression of the high-risk HPV-18 E6 in human fibrosarcoma epithelial-like HT1080 cells results in inhibition of Jak–Stat activation in response to IFN-α. This inhibitory effect, however, was not shared by the low-risk HPV-11 E6. The DNA binding and transactivation capacities of ISGF3 were impaired by HPV-18 E6 expression after stimulation with IFN-α. This coincided with an impaired tyrosine phosphorylation of Tyk2, Stat2 and Stat1. In contrast to IFN-α, tyrosine phosphorylation and DNA binding of Stat1 were not affected by HPV-18 E6 in response to IFN-γ indicating that the inhibitory effect of HPV-18 E6 on Jak–Stat activation is specific for IFN-α. These findings provided evidence for a role of HPV-18 E6 oncoprotein as a negative regulator of the Jak–Stat pathway [82].

Further analysis of the molecular mechanisms that mediate the inhibition of Tyk2 activation by HPV-18 E6 led to the identification and partial characterization of

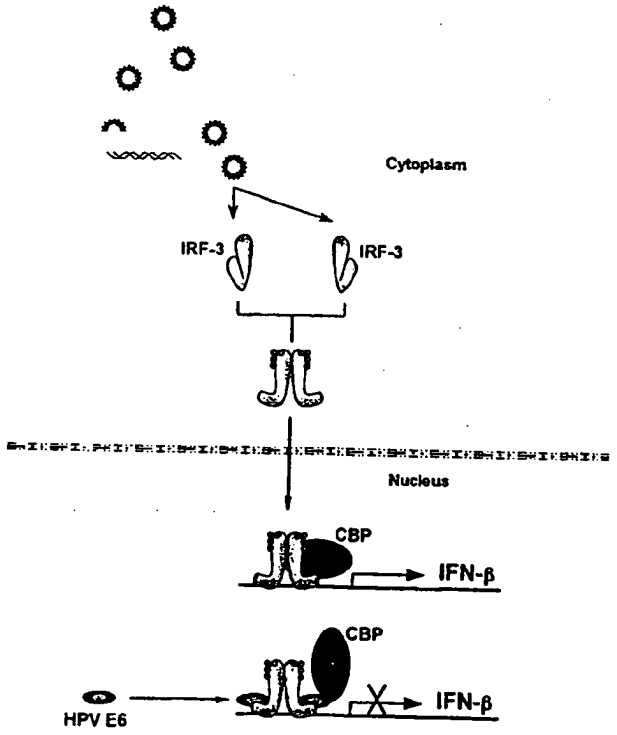

Fig. 3. Model of inhibition of IRF-3-mediated transactivation by 16-E6: IRF-3 exists in a latent state in the cytoplasm of infected cells. Virus infection leads to phosphorylation at a serine/threonine cluster within the C-terminus of the protein causing a conformational change in IRF-3 and leading to its nuclear translocation. Transcriptional activation of IFN-β promoter requires binding of IRF-3 to DNA and CBP as well as the presence of other transcriptional factors (not shown) that form the IFN-β promoter enhanceosome. The interaction of 16-E6 with IRF-3 [79] and CBP [32,33] may diminish the transactivation activities of the IRF-3/CBP complex thus decreasing IFN-β expression.

an interaction between E6 and Tyk2 [82]. Specifically, this interaction takes place preferably with both HPV-18 E6 and HPV-16 E6 and to a lesser extent with HPV-11 E6. Mapping of the interaction between the two proteins revealed that the JH_6-JH_7 domains of Tyk2, which are important for Tyk2 binding to the cytoplasmic portion of IFN-α receptor 1 chain (IFNAR1) [83,84], are also required for binding to E6 [82]. The JH_6 domain of Tyk2 contains a stretch of amino acids E–S–L–G very similar to the E–L–L/V–G sequence, which was described as an E6 interaction sequence [85]. Experiments are currently under way to determine the role of the E–S–L–G sequence in mediating Tyk2 binding to E6 and examine the function of E6-binding Tyk2 mutants in IFN-α-mediated activation of the Jak–Stat pathway. These findings allowed us to propose a model whereby the interaction of E6 with Tyk2 prevents the binding of Tyk2 to the cytoplasmic domain of IFNAR1 and the subsequent Tyk2 activation upon IFN-α stimulation (Fig. 4). Currently, the question rises as to whether Tyk2 inactivation is mediated by the ability of E6 to target proteins to proteasome dependent proteolysis. Although E6 does not affect the protein levels of Tyk2 in vitro (unpublished observations), specific degradation of phosphorylated (i.e. activated) form of Tyk2 in vivo is a possibility that is being examined.

The inactivation of Tyk2 by E6 is not the only mechanism utilized by the high-risk HPVs to block the Jak–Stat pathway. Interestingly, expression of HPV-16 E7 in the HPV-negative human epithelial cell line HaCaT was shown to inhibit the induction of IFN-α-inducible genes but had no effect on IFN-γ-inducible genes [86]. This inhibition correlated with the loss of the ISGF3 transcription complex as a result of inhibition of nuclear translocation of the IRF-9 (i.e. p48) component. This impaired nuclear translocation is most likely mediated by a direct interaction between E7 and IRF-9 (Fig. 5). Binding to IRF-9 requires the amino acids 17–37 of E7, a domain that includes the binding site to the Rb protein. Based on these data Barnard and McMillan [86] proposed that the ratio of E7 to IRF-9 may play a role in determining an effective response to IFN-α. That is, a patient with higher levels of IRF-9, or lower levels of E7, may be more likely to respond to IFN-α treatment. This notion is consistent with other observations that activation of ISGF3 is diminished in a number of HPV-positive cell lines [87]. Also, patients that do not respond to IFN-α treatment for HPV-positive condylomas have higher levels of E7 mRNA than those who are able to respond [70]. It would be of interest, however, to examine whether low-risk E7 also binds to IRF-9 with the same affinity as the high-risk protein, and whether the E7/IRF-9 interaction sufficiently accounts for the loss of ISGF3 formation in response to IFN-α.

The ability of E7 to interfere with IFN signaling was further demonstrated by its ability to bind to and inactivate the transcription factor IRF-1 [88,89]. IRF-1

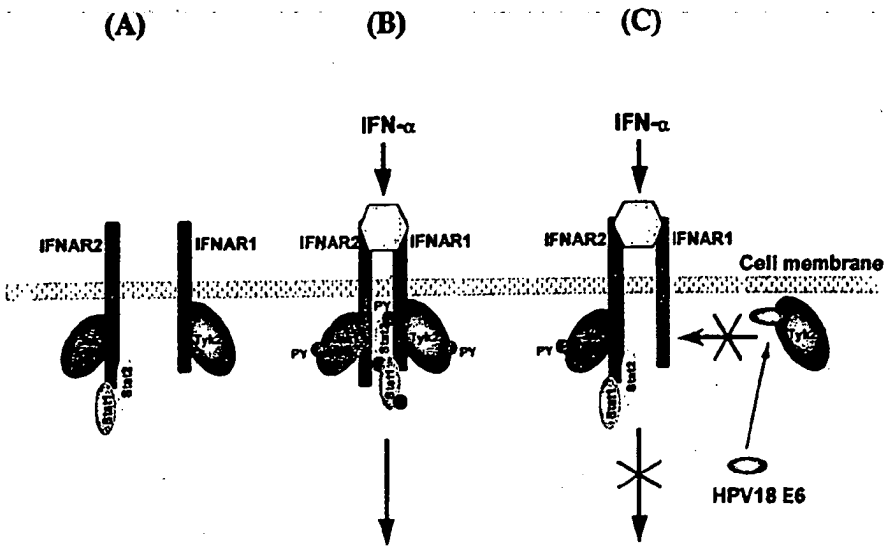

Fig. 4. Model of inhibition of Jak–Stat signaling by 18-E6: Binding of IFN-α to the receptor (A) leads to a cascade of tyrosine phosphorylation of Jak1, Tyk2, Stat2 and Stat1 molecules and (B) as explained in Fig. 2. The interaction of HPV-18 E6 with the JH_6-JH_7 domains of Tyk2 [82] may interfere with the association of Tyk2 with the cytoplasmic portion of the IFNAR1 thus preventing Tyk2 activation and phosphorylation of Stats.

was originally identified as an IFN-β promoter binding transcription factor and characterized as a critical mediator of IFN signaling induced by virus infection or IFN treatment [90]. Interestingly, IRF-1 overexpression inhibits cell growth and the introduction of activated c-Ha-ras oncogene alone is sufficient to transform embryo fibroblasts from IRF-1 knock out mice [90]. These data suggested that IRF-1 is a tumor suppressor gene product associated with the anti-proliferative effects of IFNs. Both HPV-16 and HPV-11 E7 proteins impair the transactivation activity of IRF-1 and this effect is mediated by a direct interaction between E7 and IRF-1 [88]. Binding of E7 to IRF-1 requires the Rb-binding portion of E7 and the carboxyl-terminal transactivation domain of IRF-1 [88]. The inhibition of IRF-1-dependent transactivation is most likely mediated by the recruitment of histone deacetylase (HDAC) by E7 to the IFN-β promoter (Fig. 6) [88]. The functional inactivation of IRF-1 by high and low risk E7 proteins could play an important role in the inhibition of IFN-β gene expression during viral infection. Inasmuch as both IRF-1 and p53 are required for the transcriptional activation of the cdk inhibitor p21 [91], their inactivation in HPV infected cells may represent major mechanisms that contribute into tumor formation and cervical carcinogenesis.

It is possible that HPVs has developed distinct mechanisms to block IFN action and the establishment of an antiviral state during early- or late-phase of infection. The results obtained in transformed cell lines with stable expression of E6 and/or E7 likely reflect conditions with prolonged or chronic viral infection and may not be accurate models of initial HPV infection. This notion has been supported by a recent study describing the global changes in gene expression in human keratinocytes latently infected with the high-risk HPV 31 using microarray analysis [92]. In this study, genes whose expression was repressed 2 fold or more by HPV proteins were listed into three groups. The first group consisted of regulators of cell growth such as p21, Mad, transgelin; the second group contained keratinocyte-specific genes including SprII, a small proline-rich protein found in UV-irradiated keratinocytes, and defensin; the third group consisted of IFN-inducible genes including Stat1 and 2′-5′ oligoA synthase [92]. Since Stat1 plays a crucial role in IFN-inducible gene transcription, the low basal level of its expression in HPV31 cells may account for the low level of various IFN-inducible genes and contribute to the impaired response to IFN signaling in HPV31 infected cells [92]. It would be important, however, to know which of the HPV-31 gene products are responsible for the transcriptional repression of Stat1, the mechanism of action, and whether Stat1 transcriptional repression is observed with low-risk HPVs.

10. Concluding remarks

HPVs have developed effective strategies to evade immune surveillance by downregulating both the production and action of IFNs. At the clinical level the disease frequently recurs when IFN therapy is discontinued, and the effect of IFNs is restricted to an anti-

proliferative rather than to an anti-viral or immunostimulatory action. Despite this unfavorable outcome, studies on the regulation of IFN pathways by HPV infection at the molecular level have provided and will continue to provide important insights into complex interactions between the viral oncoproteins and host factors that control innate immunity. The resulting knowledge will be useful in the design of novel strategies that combat HPV infection and associated disease. For example, a better understanding of the molecular mechanisms of inhibition of IRF-1, IRF-9 or Tyk2 by the HPV oncoproteins may lead to the development of therapies based on peptides that block the viral-host protein interactions and restore IFN signaling through endogenous control mechanisms. Another possibility is the development of anticancer therapies based on the ability of HPV infected cells to subvert IFN treatment. Significantly, the use of viruses with mild or arymptomatic infections in humans as antitumor agents is now under clinical experimentation [93–97]. Interestingly, it has been recently demonstrated that infection of various cancer cells with vesicular stomatitis virus leads to a selective killing of cells with a defective response to IFNs [98]. This may represent a new strategy for the treatment of IFN-non-responsive tumors. Whether this is a suitable treatment of HPV-associated tumorigenesis is an intriguing possibility that remains to be examined.

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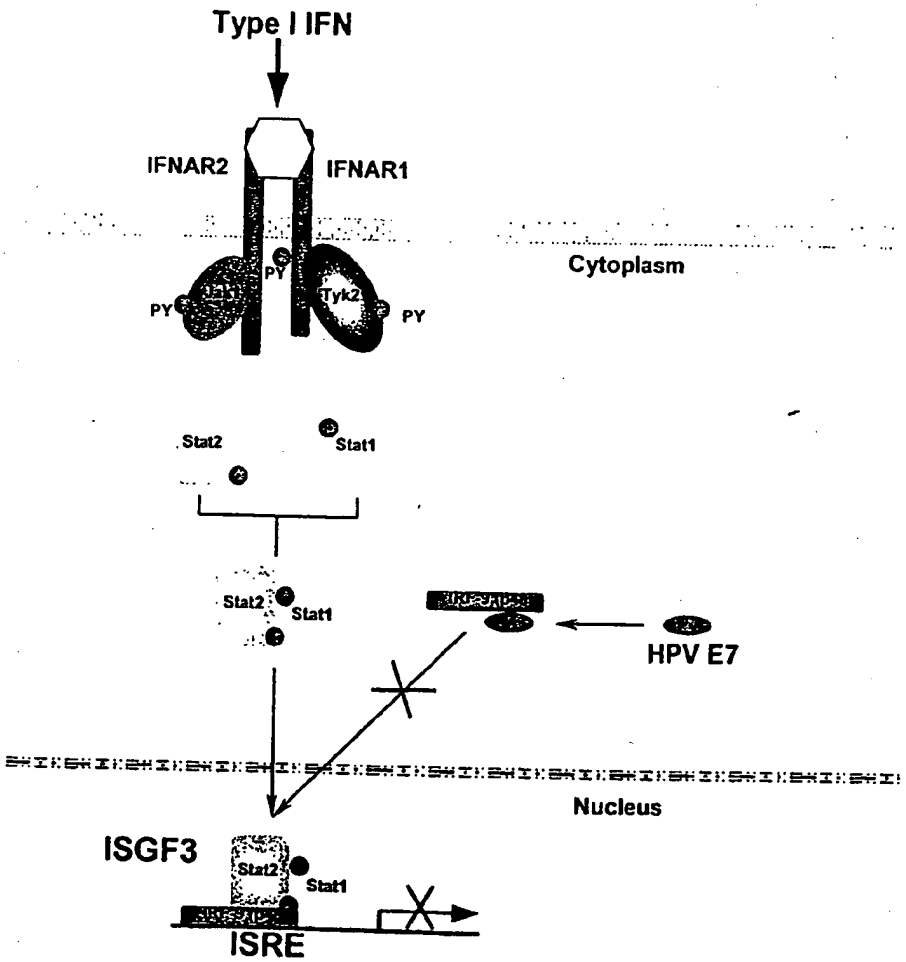

Fig. 5. Inhibition of IFN-α-induced ISGF3-dependent transcription by 16-E7: Transcriptional activation by IFN-α requires the formation of the ISGF3 complex, which consists of Stat1/Stat2 hetrodimers and IRF-9 (i.e. p48). HPV-16 E7 has been shown to bind to IRF-9 and inhibit its nuclear translation thus diminishing the DNA-binding and transactivation activities of ISGF3 [86].

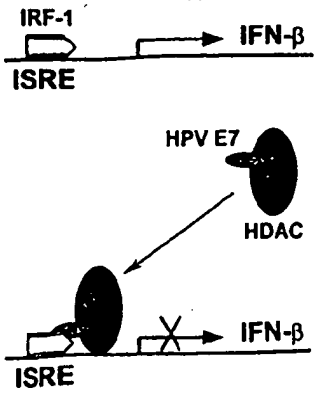

Fig. 6. Schematic model of IRF-1 inactivation by E7: Binding of IRF-1 to ISRE in the promoter of IFN-β and other IFN-inducible genes leads to transcriptional activation. HPV-E7 interacts with both IRF-1 and HDAC on the IFN-β gene promoter. Since HDAC mediates histone deacytelation of chromatin, such interactions were proposed to be responsible for the transcriptional repression of IRF-1 [88].

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